PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 21/00, 21/04	A1	(11) International Publication Number: WO 97/11086 (43) International Publication Date: 27 March 1997 (27.03.97)

(21) International Application Number: PCT/US96/15088

(22) International Filing Date: 20 September 1996 (20.09.96)

(30) Priority Data:
08/532,390 22 September 1995 (22.09.95) US

(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors: SEED, Brian; Apartment 5J, Nine Hawthorn Place, Boston, MA 02114 (US). HAAS, Jurgen; Huberweg 13, D-69198 Schriesheim (DE).

(74) Agent: LECH, Karen, F.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HIGH LEVEL EXPRESSION OF PROTEINS

(57) Abstract

The invention features a synthetic gene encoding a protein normally expressed in a mammalian cell or eukaryotic cell wherein at least one non-preferred or less preferred codon in the natural gene encoding the mammalian protein has been replaced by a preferred codon encoding the same amino acid.

```
  1  GAATTCACGC GTAAGCTTGC CGCCACCATG GTGAGCAAGG GCGAGGAGCT
 51  GTTCACCGGG GTGGTGCCCA TCCTGGTCGA GCTGGACGGC GACGTGAACG
101  GCCACAAGTT CAGCGTGTCC GGCGAGGGCG AGGGCGATGC CACCTACGGC
151  AAGCTGACCC TGAAGTTCAT CTGCACCACC GGCAAGCTGC CCGTGCCCTG
201  GCCCACCCTC GTGACCACCT TCAGCTACGG CGTGCAGTGC TTCAGCCGCT
251  ACCCCGACCA CATGAAGCAG CACGACTTCT TCAAGTCCGC CATGCCCGAA
301  GGCTACGTCC AGGAGCGCAC CATCTTCTTC AAGGACGACG GCAACTACAA
351  GACCCGCGCC GAGGTGAAGT TCGAGGGCGA CACCCTGGTG AACCGCATCG
401  AGCTGAAGGG CATCGACTTC AAGGAGGACG GCAACATCCT GGGGCACAAG
451  CTGGAGTACA ACTACAACAG CCACAACGTC TATATCATGG CCGACAAGCA
501  GAAGAACGGC ATCAAGGTGA ACTTCAAGAT CCGCCACAAC ATCGAGGACG
551  GCAGCGTGCA GCTCGCCGAC CACTACCAGC AGAACACCCC CATCGGCGAC
601  GGCCCCGTGC TGCTGCCCGA CAACCACTAC CTGAGCACCC AGTCCGCCCT
651  GAGCAAAGAC CCCAACGAGA AGCGCGATCA CATGGTCCTG CTGGAGTTCG
701  TGACCGCCGC CGGGATCACT CACGGCATGG ACGAGCTGTA CAAGTAAAGC
751  GGCCGCGGAT CC    (SEQ ID NO: 40)
```

What is claimed is:

1. A synthetic gene encoding a protein normally expressed in a eukaryotic cell wherein at least one non-preferred or less preferred codon in the natural gene encoding said protein has been replaced by a preferred codon encoding the same amino acid.

2. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least 110% of that expressed by said natural gene in an in vitro mammalian cell culture system under identical conditions.

3. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least 150% of that expressed by said natural gene in an in vitro cell culture system under identical conditions.

4. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least 200% of that expressed by said natural gene in an in vitro cell culture system under identical conditions.

5. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least 500% of that expressed by said natural gene in an in vitro cell culture system under identical conditions.

6. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least ten times that

expressed by said natural gene in an <u>in vitro</u> cell culture system under identical conditions.

7. The synthetic gene of claim 1 wherein at least 10% of the codons in said natural gene are non-preferred codons.

8. The synthetic gene of claim 8 wherein at least 50% of the codons in said natural gene are non-preferred codons.

9. The synthetic gene of claim 1 wherein at least 50% of the non-preferred codons and less preferred codons present in said natural gene have been replaced by preferred codons.

10. The synthetic gene of claim 1 wherein at least 90% of the non-preferred codons and less preferred codons present in said natural gene have been replaced by preferred codons.

11. The synthetic gene of claim 1 wherein said protein is green fluorescent protein.

12. A method for preparing a synthetic gene encoding a protein normally expressed by eukaryotic cells, comprising identifying non-preferred and less-preferred codons in the natural gene encoding said protein and replacing one or more of said non-preferred and less-preferred codons with a preferred codon encoding the same amino acid as the replaced codon.

Syngp120mn

```
   1  CTCGAGATCC ATTGTGCTCT AAAGGAGATA CCCGGCCAGA CACCCTCACC
  51  TGCGGTGCCC AGCTGCCCAG GCTGAGGCAA GAGAAGGCCA GAAACCATGC
 101  CCATGGGGTC TCTGCAACCG CTGGCCACCT TGTACCTGCT GGGGATGCTG
 151  GTCGCTTCCG TGCTAGCCAC CGAGAAGCTG TGGGTGACCG TGTACTACGG
 201  CGTGCCCGTG TGGAAGGAGG CCACCACCAC CCTGTTCTGC GCCAGCGACG
 251  CCAAGGCGTA CGACACCGAG GTGCACAACG TGTGGGCCAC CCAGGCGTGC
 301  GTGCCCACCG ACCCCAACCC CCAGGAGGTG GAGCTCGTGA ACGTGACCGA
 351  GAACTTCAAC ATGTGGAAGA ACAACATGGT GGAGCAGATG CATGAGGACA
 401  TCATCAGCCT GTGGGACCAG AGCCTGAAGC CCTGCGTGAA GCTGACCCCC
 451  CTGTGCGTGA CCCTGAACTG CACCGACCTG AGGAACACCA CCAACACCAA
 501  CAACAGCACC GCCAACAACA ACAGCAACAG CGAGGGCACC ATCAAGGGCG
 551  GCGAGATGAA CAACTGCAGC TTCAACATCA CCACCAGCAT CCGCGACAAG
 601  ATGCAGAAGG AGTACGCCCT GCTGTACAAG CTGGATATCG TGAGCATCGA
 651  CAACGACAGC ACCAGCTACC GCCTGATCTC CTGCAACACC AGCGTGATCA
 701  CCCAGGCCTG GCCCAAGATC AGCTTCGAGC CCATCCCCAT CCACTACTGC
 751  GCCCCCGCCG GCTTCGCCAT CCTGAAGTGC AACGACAAGA AGTTCAGCGG
 801  CAAGGGCAGC TGCAAGAACG TGAGCACCGT GCAGTGCACC CACGGCATCC
 851  GGCCGGTGGT GAGCACCCAG CTCCTGCTGA ACGGCAGCCT GGCCGAGGAG
 901  GAGGTGGTGA TCCGCAGCGA GAACTTCACC GACAACGCCA AGACCATCAT
 951  CGTGCACCTG AATGAGAGCG TGCAGATCAA CTGCACGCGT CCCAACTACA
1001  ACAAGCGCAA GCGCATCCAC ATCGGCCCCG GGCGCGCCTT CTACACCACC
1051  AAGAACATCA TCGGCACCAT CCGCCAGGCC CACTGCAACA TCTCTAGAGC
1101  CAAGTGGAAC GACACCCTGC GCCAGATCGT GAGCAAGCTG AAGGAGCAGT
1151  TCAAGAACAA GACCATCGTG TTCAACCAGA GCAGCGGCGG CGACCCCGAG
1201  ATCGTGATGC ACAGCTTCAA CTGCGGCGGC GAATTCTTCT ACTGCAACAC
1251  CAGCCCCCTG TTCAACAGCA CCTGGAACGG CAACAACACC TGGAACAACA
1301  CCACCGGCAG CAACAACAAT ATTACCCTCC AGTGCAAGAT CAAGCAGATC
1351  ATCAACATGT GGCAGGAGGT GGGCAAGGCC ATGTACGCCC CCCCCATCGA
1401  GGGCCAGATC CGGTGCAGCA GCAACATCAC CGGTCTGCTG CTGACCCGCG
1451  ACGGCGGCAA GGACACCGAC ACCAACGACA CCGAAATCTT CCGCCCCGGC
```

FIG 1
(SHEET 1 OF 4)

1501 GGGGGCGACA TGCGCGACAA CTGGAGATCT GAGCTGTACA AGTACAAGGT

1551 GGTGACGATC GAGCCCCTGG GCGTGGCCCC CACCAAGGCC AAGCGCCGCG

1601 TGGTGCAGCG CGAGAAGCGC TAAAGCGGCC GC (SEQ ID NO:34)

FIG 1

(SHEET 2 OF 4)

Syngp160mn

1 ACCGAGAAGC TGTGGGTGAC CGTGTACTAC GGCGTGCCCG TGTGGAAGGA
51 GGCCACCACC ACCCTGTTCT GCGCCAGCGA CGCCAAGGCG TACGACACCG
101 AGGTGCACAA CGTGTGGGCC ACCCAGGCCT GCGTGCCCAC CGACCCCAAC
151 CCCCAGGAGG TGGAGCTCGT GAACGTGACC GAGAACTTCA ACATGTGGAA
201 GAACAACATG GTGGAGCAGA TGCATGAGGA CATCATCAGC CTGTGGGACC
251 AGAGCCTGAA GCCCTGCGTG AAGCTGACCC CCCTGTGCGT GACCCTCAAC
301 TGCACCGACC TGAGGAACAC CACCAACACC AACAACAGCA CCGCCAACAA
351 CAACAGCAAC AGCGAGGGCA CCATCAAGGG CGGCGAGATG AAGAACTGCA
401 GCTTCAACAT CACCACCAGC ATCCGCGACA AGATCCAGAA GGAGTACGCC
451 CTGCTGTACA AGCTGGATAT CGTGAGCATC GACAACGACA GCACCAGCTA
501 CCGCCTGATC TCCTGCAACA CCAGCGTGAT CACCCAGGCC TGCCCCAAGA
551 TCAGCTTCGA GCCCATCCCC ATCCACTACT GCGCCCCCGC CGGCTTCGCC
601 ATCCTGAAGT GCAACGACAA GAAGTTCAGC GGCAAGGGCA GCTGCAAGAA
651 CGTGAGCACC GTGCAGTGCA CCCACGGCAT CCGGCCCGTG GTGAGCACCC
701 AGCTCCTGCT GAACGGCAGC CTGGCCGAGG AGGAGGTGGT GATCCGCAGC
751 GAGAACTTCA CCGACAACGC CAAGACCATC ATCGTGCACC TGAATGAGAG
801 CGTGCAGATC AACTGCACGC GTCCCAACTA CAACAAGCGC AAGCGCATCC
851 ACATCGGCCC CGGCCGCGCC TTCTACACCA CCAAGAACAT CATCGGCACC
901 ATCCGCCAGG CCCACTGCAA CATCTCTAGA GCCAAGTGGA ACGACACCCT
951 GCGCCAGATC GTGAGCAAGC TGAAGGAGCA GTTCAAGAAC AAGACCATCG
1001 TGTTCAACCA GAGCAGCGGC GGCGACCCCG AGATCGTGAT GCACAGCTTC
1051 AACTGCGGCG GCGAATTCTT CTACTGCAAC ACCAGCCCCC TGTTCAACAG
1101 CACCTGGAAC GGCAACAACA CCTGGAACAA CACCACCGGC AGCAACAACA
1151 ATATTACCCT CCAGTGCAAG ATCAAGCAGA TCATCAACAT GTGGCAGGAG
1201 GTGGGCAAGG CCATGTACGC CCCCCCCATC GAGGGCCAGA TCCGGTGCAG
1251 CAGCAACATC ACCGGTCTGC TGCTGACCCG CGACGGCGGC AAGGACACCG
1301 ACACCAACGA CACCGAAATC TTCCGCCCCG GCGGCGGCGA CATGCGCGAC
1351 AACTGGAGAT CTGAGCTGTA CAAGTACAAG GTGGTGACGA TCGAGCCCCT
1401 GGGCGTGGCC CCCACCAAGG CCAAGCGCCG CGTGGTGCAG CGCGAGAAGC

FIG. 1
(SHEET 3 OF 4

1451 GGGCCGCCAT CGGCGCCCTG TTCCTGGGCT TCCTGGGGGC GGCGGGCAGC
1501 ACCATGGGGG CCGCCAGCGT GACCCTGACC GTGCAGGCCC GCCTGCTCCT
1551 GAGCGGCATC GTGCAGCAGC AGAACAACCT CCTCCGCGCC ATCGAGGCCC
1601 AGCAGCATAT GCTCCAGCTC ACCGTGTGGG GCATCAAGCA GCTCCAGGCC
1651 CGCGTGCTGG CCGTGGAGCG CTACCTGAAG GACCAGCAGC TCCTGGGCTT
1701 CTGGGGCTGC TCCGGCAAGC TGATCTGCAC CACCACGGTA CCCTGGAACG
1751 CCTCCTGGAG CAACAAGAGC CTGGACGACA TCTGGAACAA CATGACCTGG
1801 ATGCAGTGGG AGCGCGAGAT CGATAACTAC ACCAGCCTGA TCTACAGCCT
1851 GCTGGAGAAG AGCCAGACCC AGCAGGAGAA GAACGAGCAG GAGCTGCTGG
1901 AGCTGGACAA CTGGGCGAGC CTGTGGAACT GGTTCGACAT CACCAACTGG
1951 CTGTGGTACA TCAAAATCTT CATCATGATT GTGGGCGGCC TGGTGGGCCT
2001 CCGCATCGTG TTCGCCGTGC TGAGCATCGT GAACCGCGTG CGCCAGGGCT
2051 ACAGCCCCCT GAGCCTCCAG ACCCGGCCCC CCGTGCCGCG CGGGCCCGAC
2101 CGCCCCGAGG GCATCGAGGA GGAGGGCGGC GAGCGCGACC GCGACACCAG
2151 CGGCAGGCTC GTGCACGGCT TCCTGGCGAT CATCTGGGTC GACCTCCGCA
2201 GCCTGTTCCT GTTCAGCTAC CACCACCGCG ACCTGCTGCT GATCGCCGCC
2251 CGCATCGTGG AACTCCTAGG CCGCCGCGGC TGGGAGGTGC TGAAGTACTG
2301 GTGGAACCTC CTCCAGTATT GGAGCCAGGA GCTGAAGTCC AGCGCCGTGA
2351 GCCTGCTGAA CGCCACCGCC ATCGCCGTGG CCGAGGGCAC CGACCGCGTG
2401 ATCGAGGTGC TCCAGAGGGC CGGGAGGGCG ATCCTGCACA TCCCCACCCG
2451 CATCCGCCAG GGGCTCGAGA GGGCGCTGCT G (SEQ ID NO:35)

FIG. 1
(SHEET 4 OF 4)

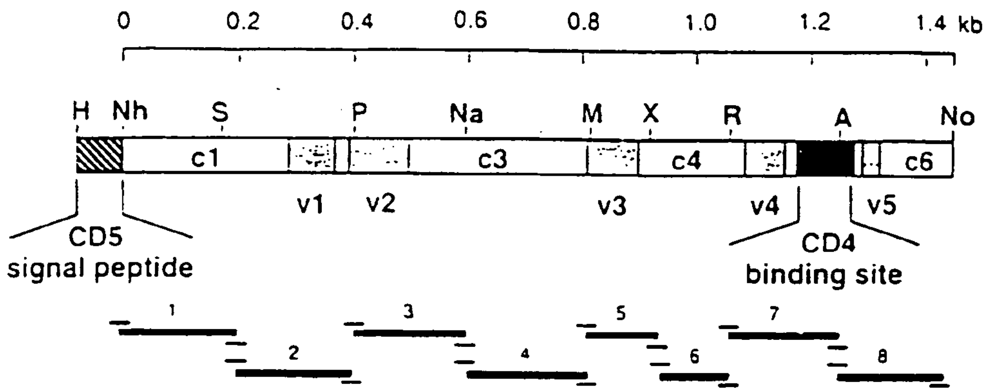

0
0.2
0.4
0.6
0.8
1.0
1.2
1.4 kb
H
Nh
S
P
Na
M
X
R
A
No
c1
c3
c4
c6
v1
v2
v3
v4
v5
CD5
signal peptide
CD4
binding site
1
2
3
4
5
6
7
8

FIGURE 2

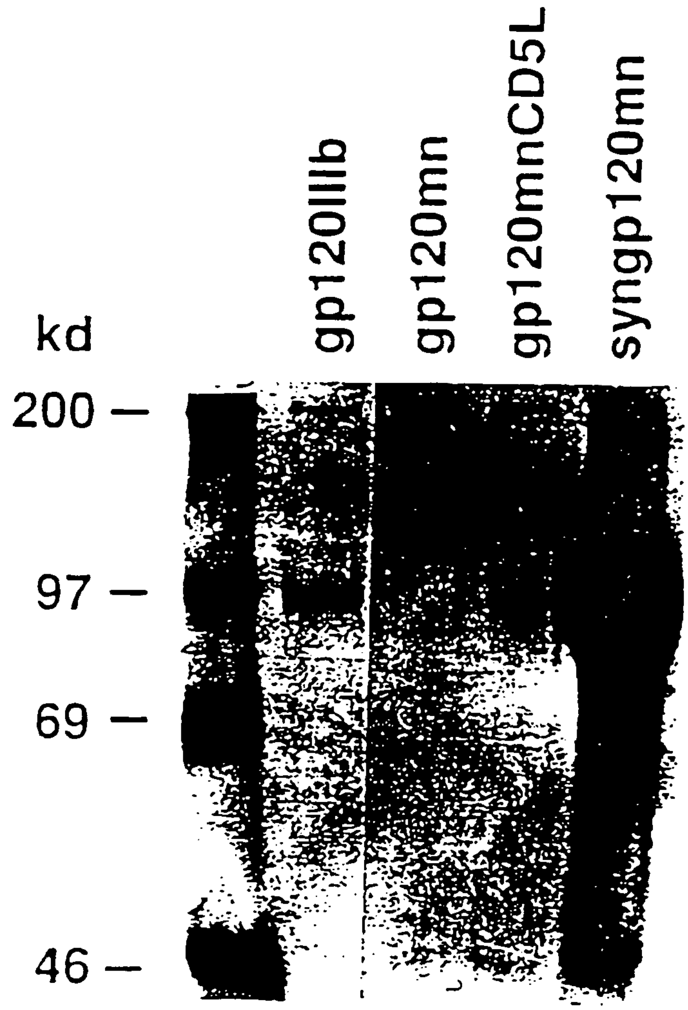


FIGURE 3

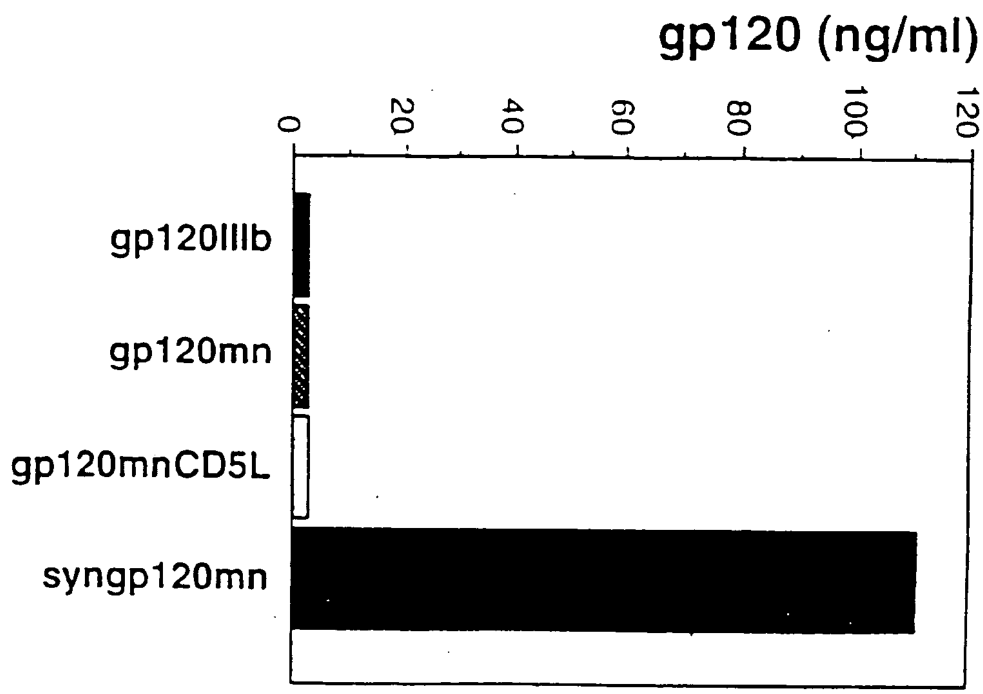

gp120 (ng/ml)
0
20
40
60
80
100
120
gp120IIIb
gp120mn
gp120mnCD5L
syngp120mn

FIGURE 4

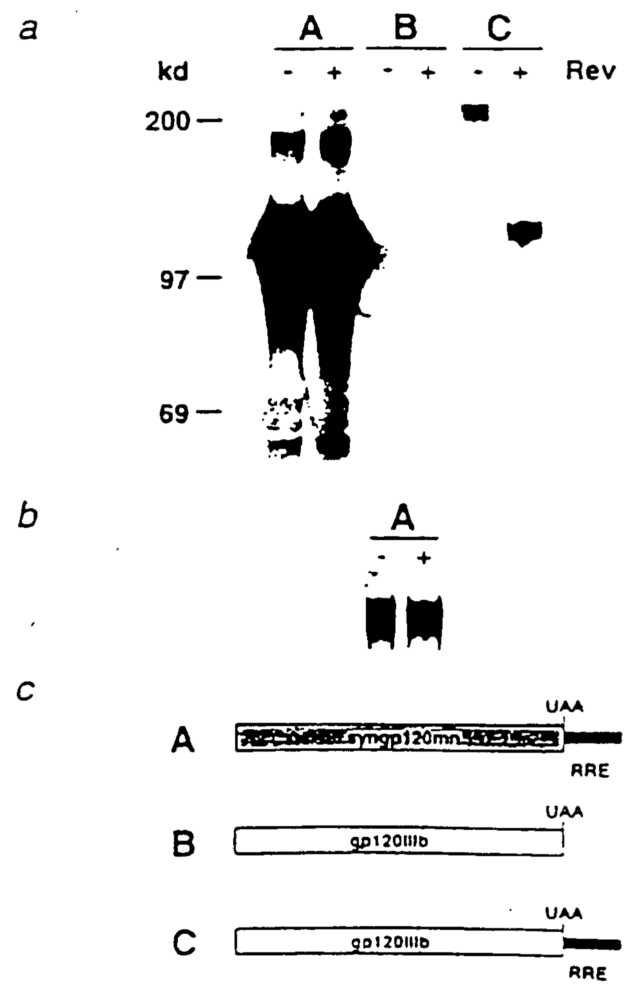

a
A
B
C
kd
- + - + - +
Rev
200
97
69
b
A
- +
c
A
UAA
RRE
B
UAA
gp120IIIb
C
UAA
gp120IIIb
RRE

FIGURE 5

```
                              M   N   P   V   I   S   I   T   L   L   L   S   V   L   Q   M   S   R   G   Q
(SEQ ID NO:36) env——>atg aat cca gta ata agt ata aca tta tta tta agt gta tta caa atg agt aga gga caa
(SEQ ID NO:37) wt——> atg aac cca gtc atc agc atc act ctc ctg ctt tca gtc ttg cag atg tcc cga gga cag

                      R   V   I   S   L   T   A   C   L   V   N   Q   N   L   R   L   D   C   R   H
               env   aga gta ata agt tta aca gca tgt tta gta aat caa aat ttg aga tta gat tgt aga cat
               wt    agg gtg atc agc ctg aca gcc tgc ctg gtg aa  cag aac ctt cga ctg gac tgc cgt cat

                      E   N   N   T   N   L   P   I   Q   H   E   F   S   L   T   R   E   K   K   K
               env   gaa aat aat aca cct ttg cca ata caa cat gaa ttt tca tta acg cgt gaa aaa aaa aaa
               wt    gag aat aac acc aac ttg ccc atc cag cat gag ttc agc ctg acc cga gag aag aag aag

                      H   V   L   S   G   T   L   G   V   P   E   H   T   Y   R   S   R   V   N   L
               env   cat gta tta agt gga aca tta gga gta cca gaa cat aca tat aga agt aga gta aat ttg
               wt    cac gtg ctg tca ggc acc ctg ggg gtt ccc gag cac act tac cgc tcc cgc gtc aac ctt

                      F   S   D   R   F   I   K   V   L   T   L   A   N   F   T   T   K   D   E   G
               env   ttt agt gat aga ttc ata aaa gta tta aca tta gca aat ttt aca aca aaa gat gaa gga
               wt    ttc agt gac cgc ttt atc aag gtc ctt act cta gcc aac ttc acc acc aag gat gag ggc

                      D   Y   M   C   E   L   R   V   S   G   Q   N   P   T   S   S   N   K   T   I
               env   gat tat atg tgt gag ctc aga gta agt gga caa aat cca aca agt agt aat aaa aca ata
               wt    gac tac atg tgt gaa ctt cga gtc tcg ggc cag aat ccc aca agc tcc aat aaa act atc

                      N   V   I   R   D   K   L   V   K   C   G   G   I   S   L   L   V   Q   N   T
               env   aat gta ata aga gat aaa tta gta aaa tgt gga gga ata agt tta tta gta caa aat aca
               wt    aat gtg atc aga gac aag ctg gtc aag tgt ggt ggc ata agc ctg ctg gtt caa aac act

                      S   W   L   L   L   L   L   L   S   L   S   F   L   Q   A   T   D   F   I   S
               env   agt tgg tta tta tta tta tta tta agt tta agt ttt tta caa gca aca gat ttt ata agt
               wt    tcc tgg ctg ctg ctg ctc ctg ctt tcc ctc tcc ttc ctc caa gcc acg gac ttc att tct

                      L   •
               env   tta tga
               wt    ctg tga
```

FIGURE 6

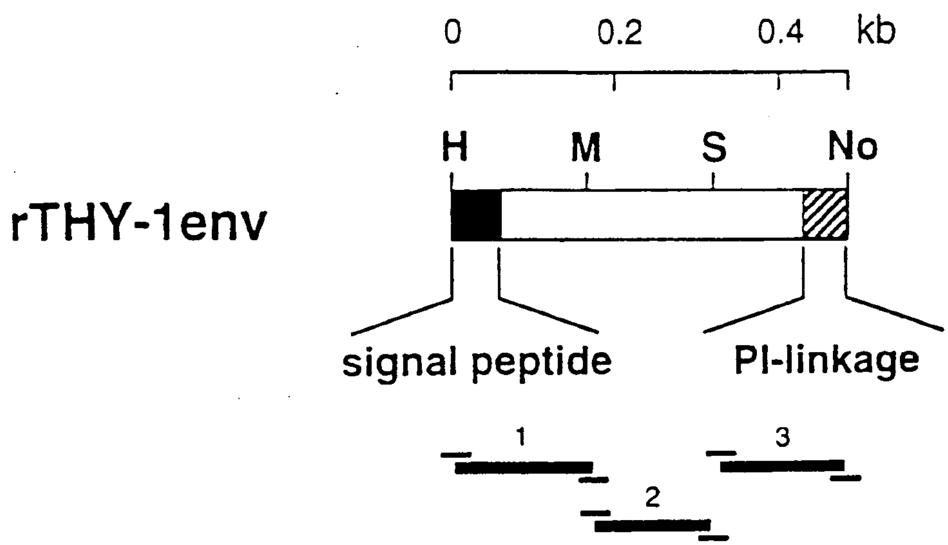


FIGURE 7

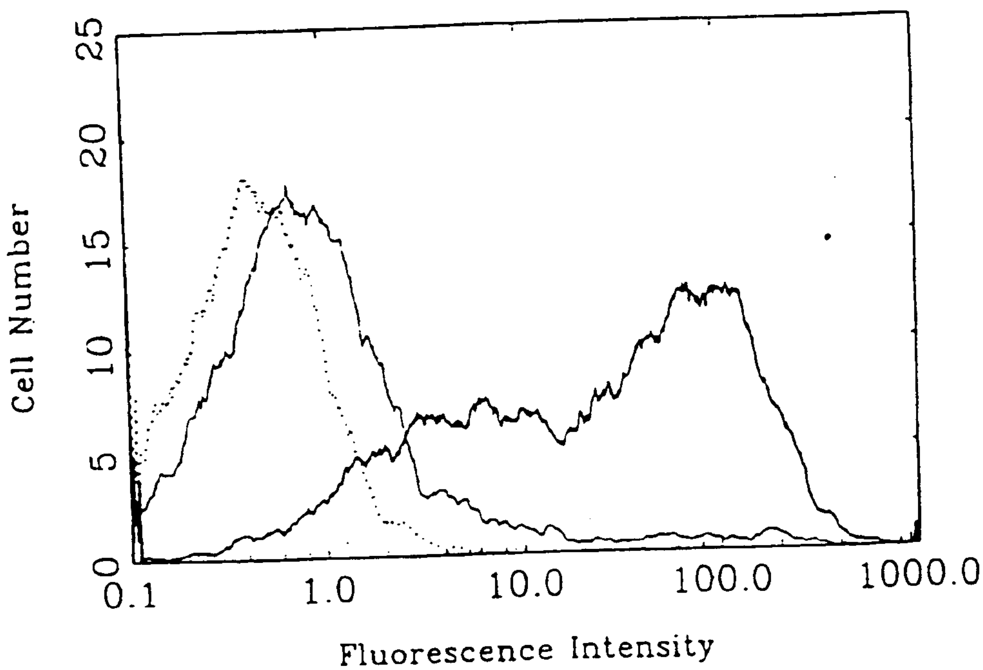

Cell Number
0
5
10
15
20
25
0.1
1.0
10.0
100.0
1000.0
Fluorescence Intensity

FIGURE 8

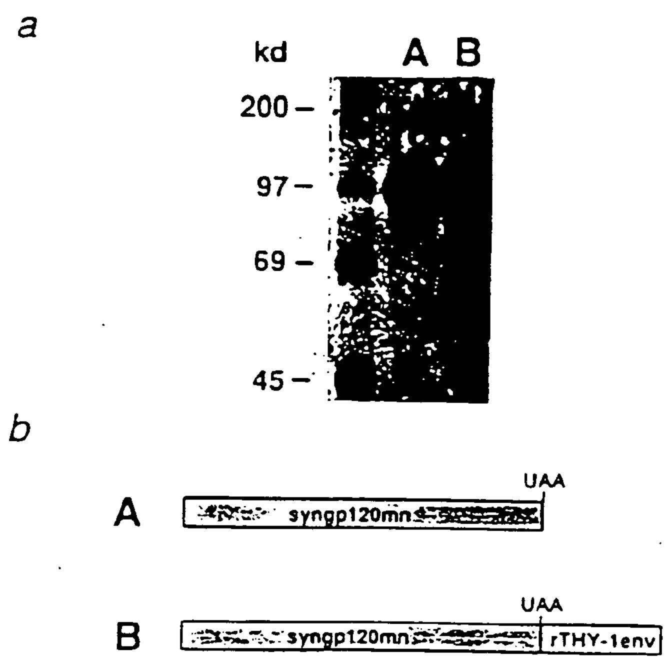
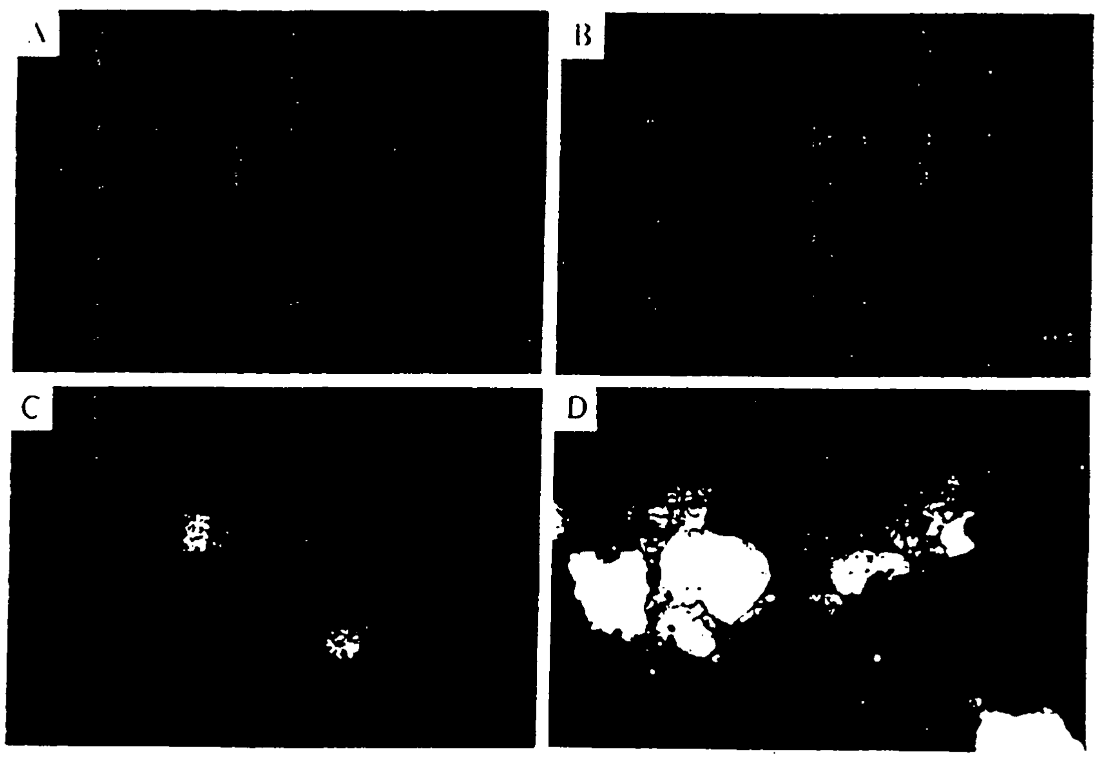

a
kd
A
B
200
97
69
45
b
A
UAA
syngp120mn
B
UAA
syngp120mn
rTHY-1env

FIGURE 9

FIG. 10



1 GAATTCACGC GTAAGCTTGC CGCCACCATG GTGAGCAAGG GCGAGGAGCT
51 GTTCACCGGG GTGGTGCCCA TCCTGGTCGA GCTGGACGGC GACGTGAACG
101 GCCACAAGTT CAGCGTGTCC GGCGAGGGCG AGGGCGATGC CACCTACGGC
151 AAGCTGACCC TGAAGTTCAT CTGCACCACC GGCAAGCTGC CCGTGCCCTG
201 GCCCACCCTC GTGACCACCT TCAGCTACGG CGTGCAGTGC TTCAGCCGCT
251 ACCCCGACCA CATGAAGCAG CACGACTTCT TCAAGTCCGC CATGCCCGAA
301 GGCTACGTCC AGGAGCGCAC CATCTTCTTC AAGGACGACG GCAACTACAA
351 GACCCGCGCC GAGGTGAAGT TCGAGGGCGA CACCCTGGTG AACCGCATCG
401 AGCTGAAGGG CATCGACTTC AAGGAGGACG GCAACATCCT GGGGCACAAG
451 CTGGAGTACA ACTACAACAG CCACAACGTC TATATCATGG CCGACAAGCA
501 GAAGAACGGC ATCAAGGTGA ACTTCAAGAT CCGCCACAAC ATCGAGGACG
551 GCAGCGTGCA GCTCGCCGAC CACTACCAGC AGAACACCCC CATCGGCGAC
601 GGCCCCGTGC TGCTGCCCGA CAACCACTAC CTGAGCACCC AGTCCGCCCT
651 GAGCAAAGAC CCCAACGAGA AGCGCGATCA CATGGTCCTG CTGGAGTTCG
701 TGACCGCCGC CGGGATCACT CACGGCATGG ACGAGCTGTA CAAGTAAAGC
751 GGCCGCGGAT CC (SEQ ID NO: 40)

FIG. 11

INTERNATIONAL SEARCH REPORT

Inter. ..onal application No.
PCT/US96/15088

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/00, 21/04
US CL :536/23.1, 23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, Medline, Biosis, Embase, Scisearch, WPIDS, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOLLER et al. HIV1 Integrase Expressed in Escherichia coli From a Synthetic Gene. Gene. 1993, Vol.136, pages 323-328, especially pages 323-327.	1-10, 12
X	SCORER et al. The Intracellular Production and Secretion of HIV-1 Envelope Protein in the Methylotrophic Yeast Pichia pastoris. Gene. 1993, Vol.136, pages 111-119, especially pages 111-118.	1-10, 12
X	HERNAN et al. Human Hemoglobin Expression in Escherichia coli: Importance of Optimal Codon Usage. Biochemistry. 1992, Vol.31, pages 8619-8628, especially pages 8619-8627.	1-10, 12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
26 NOVEMBER 1996	23 JAN 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	ENRIQUE D. LONGTON Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15088

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WILLIAMS et al. Design, Synthesis and Expression of a Human Interleukin-2 Gene Incorporating the Codon Usage Bias Found in Highly Expressed Escherichia coli Genes. Nucleic Acids Research. 1988, Vol.16, No.22, pages 10453-10467, especially pages 10453-10466.	1-10, 12 ---- 11
X	RANGWALA et al. High-Level Production of Active HIV-1 Protease In Escherichia coli. Gene. 1992, Vol.122, pages 263-269, especially pages 263-268.	1-10, 12
P, X	US 5,464,774 A (BAIRD et al.) 07 November 1995 (07/11/95), see entire document, especially insert at top of columns 13 and 14; column 7, lines 27-51.	1-10, 12
Y	INOUYE et al. Aequirea Green Fluorescent Protein Expression of the Gene and Fluorescence Characteristics of the Recombinant Protein. FEBS Letters. 1994, Vol.341, pages 277-280, especially pages 277-279.	11